The comparison effect of the leaves of three indigenous plants of Senegal (*Crataeva religiosa* Forts, *Azadirachta indica* A Juss and *Senna occidentalis* L.) on the external forms of *Callosobruchus maculatus*, the main devastator of cowpea seeds (*Vigna unguiculata* Walp.)

Ablaye Faye[1], Toffene Diome[1], Cheikh Thiaw[2], Mbacké Sembène[1,3]

[1]*Department of Animal Biology, Faculty of Sciences and Technology, University Cheikh Anta Diop of Dakar, B.P. 5005, Dakar, Senegal*
[2]*ISRA/ CNRA Bambey, Senegal*
[3]*Laboratory BioPASS CBGP, IRD, Bel Air B.P.1386 Dakar, Senegal*

Abstract: *Comparing the efficacy of contact of the leaves of three plants (Crataeva religiosa, Azadirachta indica and Senna occidentalis) on the most formidable devastator of stored cowpea seeds, Callosobruchus maculatus was reviewed in our study. Thus, the crushed leaves of these plants were impacted on the external forms of this insect (eggs and adults). With the contact of all plants, Eggs are sensitive, but their sensitivity is increased with A. indica and less important with S. occidentalis. We observe this with any impacted dose. Adults showed the same sensitivity patterns as eggs with the two lowest doses (D_1 (0.00273g / cm^3) and D_2 (0.00546g / cm^3)), but with higher doses (D_3 (0.0109g / cm^3) and D_4 (0.021g / cm^3)), C. religiosa lays the pawns. The adults of this insect are extremely sensitive to the contact with the crushed leaves of this plant, which induced 100% mortalities from the 12th d (D_4 (0.021g / cm^3).) To combine leaf shreds of these plants would be an effective means of controlling C maculatus in farmers' actual cowpea storage systems.*

Key words: *Callosobruchus maculatus, adults, eggs, crushed leaves, Crataeva religiosa, Azadirachta indica, Senna occidentalis*

I. Introduction:

The damage caused by *Callosobruchus maculatus* on cowpea seeds has long worriedthe farmers about how to store this commodity, which is very important to them. Damage caused by *C. maculatus* larvae affects the quality, thequantity, and the commercial and agronomic values of stored cowpeas [1]. Losses in stock can be estimated at more than 800 g / kg of seeds after 7 months of storage, and these ones are indestructible[6]. The destruction of stored cowpea is aggravated by the placement of opportunistic insects in the stocks, increasing the effect of *C. maculatus* on cowpea seeds. To this end, farmers use synthetic insecticides, which often cause environmental degradation and poisoning of animal and human populations.

To counter these effects, several control methods have been highlighted by farmers and scientists ([2], [5], [11]) to combat the destructive effect of cowpea Insect pests.

It is in this context that we propose to judge the compared effectiveness of the contact of the leaves of three plants (*Crataeva religiosa*, *Azadirachta indica* and *Senna occidentalis*) on the mortality of eggs and adults of *C. maculatus*. The choice of these plants is motivated by their use in several scientific studies aiming to limit the damage caused by insect pests on stored products ([3], [12], [7]) and their availability to the farmers.

II. Material and methods

II.1. Collection and conservation

The *C. maculatus* strain used in the experiment was obtained from a sample of infected cowpea bought at the Sandiara weekly market (Department of Mbour, Senegal). Cowpeas used for livestock had also been bought in the same market. These cowpea seeds were taken to the Entomology and Acarology laboratory of the Faculty of Science and Technology

of Cheikh Anta Diop Dakar University where they were packed and stored in the freezer for 96 hours to eliminate any hidden infestation. The seeds were then placed in glass jars of 16 cm high and 8 cm in diameter hermetically sealed to prevent any possible further infestation.
The plant organs used are leaves of *C. religiosa*, *A. indica* and *S. occidentalis*. These leaves were harvested early in the morning before sunrise to obtain a high concentration of the active substances within it. The harvest was carried out in January and February. After harvest, these leaves were immediately used for contact tests.

II.2. Mass breeding

Pea beetles were raised in laboratory. The breeding consisted in maintaining the strain in order to obtain a sufficient number of individuals to ensure the tests. This breeding was carried out in cylindrical jars made of rubber, 16 cm in diameter and 8 cm high. In each jar cowpea seeds are introduced until its base is completely hidden and a sufficient number of male and female insects are present. After 24 hours of contact with the seeds, the weevils were recovered. Infested seeds were monitored and adults emerging from them were used either for adulticidal testing or to maintain mass breeding. In this way the strain of *C. maculatus* was conserved in the laboratory.

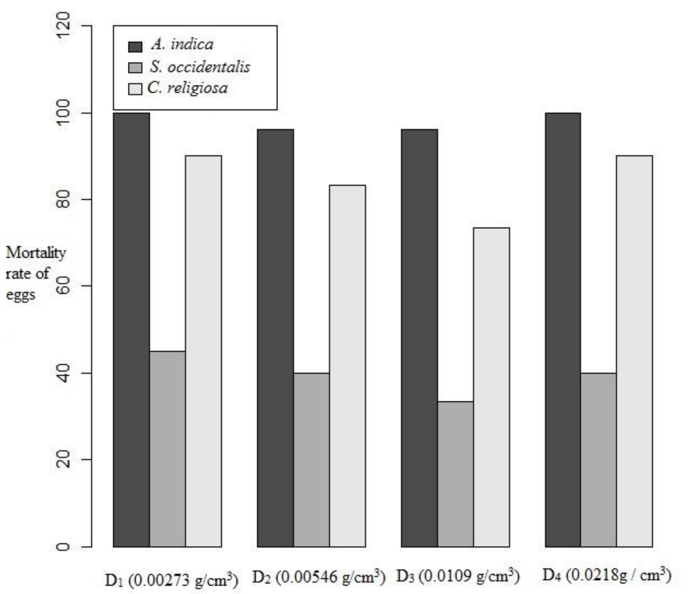
II.3. Methodology

The leaves were then crushed with mortar and placed in jars of 10.5 cm in diameter and 8 cm in height, the lids of which were screened. In each jar, 12 non-sexuous *C. maculatus* adults aged up to 48 hours were introduced. We used four weights of fresh leaves (2g, 4g, 8g and 16g) for each plant. For each weight used, three repetitions were made and a white light always accompanies the repetitions. For each repetition, the crushed leaves and insects were mixed in the same jar. Dead beetles were counted at 24-hour intervals. All the pea beetle that didn't make any movement when touched on legs and the antennas, were counted dead. Mortalities were recorded daily.
Ovicidal tests were carried out in the same jars as before and the same plant leaf weights were used. After grinding, the fresh leaves are mixed with cowpea seeds, each bearing an egg, in the order of 12 seeds per jar. For each weight used, three repetitions were performed and a white light always accompanies them. At the end of the experiment, we had a count of the hatched eggs and the unhatched eggs. This takes place 2 weeks after the introduction of crushed leaves and seeds carrying an egg into the jars. This procedure allowed us to calculate the embryonic mortality rate by the following formula:
ME = (Number of unhatched eggs) / (Total egg number) * 100
This parameter was reported as a percentage and corrected by the following Abbott formula (1925):
Mc = (MT-MTo) / (100-MTo) * 100
Mc = corrected mortality, MTo = observed mortality, MT = control mortality.
The same formula was also used to quantify the adult mortality rate. Statistical analyzes (ANOVA tests) had been carried out by software R. The results will be presented in graphic form.

III. Results

III.1. Ovicidal Effect

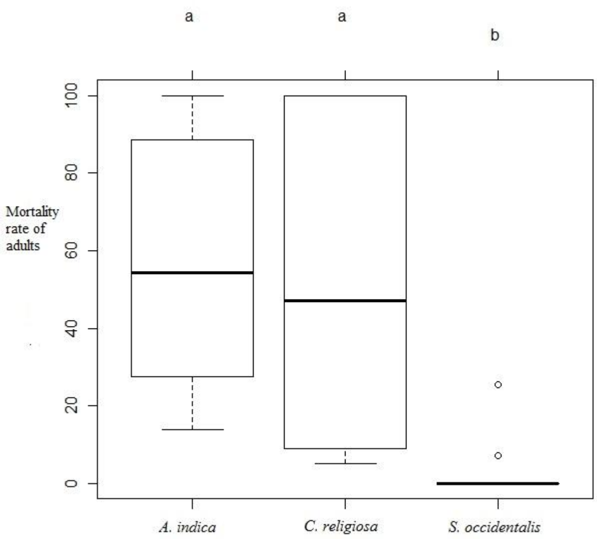
Comparingthe effect of the contact of fresh ground leaves of the three plants (*A. indica*, *S. occidentalis* and *C. religiosa*) on the eggs of *C. maculatus*, we notice a differential efficacy according to the plants and the doses. Thus, *A. indica* was found to be more effective than the two other plants used (*S. occidentalis* and *C. religiosa*) with mortalities greater than 95% for all doses. *S. occidentalis* induced the lowest mortalities exceeding less than 45%. We find that all the plants gave the same rate of mortality according to the doses. Thus, we note that the lowest dose and the highest dose have both given the same mortality for all plants except *S. occidentalis*. The variance analysis of the ovicidal activity of fresh ground leaves of the different plants revealed a highly significant difference as a function of the plants applied (p <0.0001).



Comparison of mortalities induced by the contact of crushed fresh leaves of the three plants on the eggs of *C. maculatus*

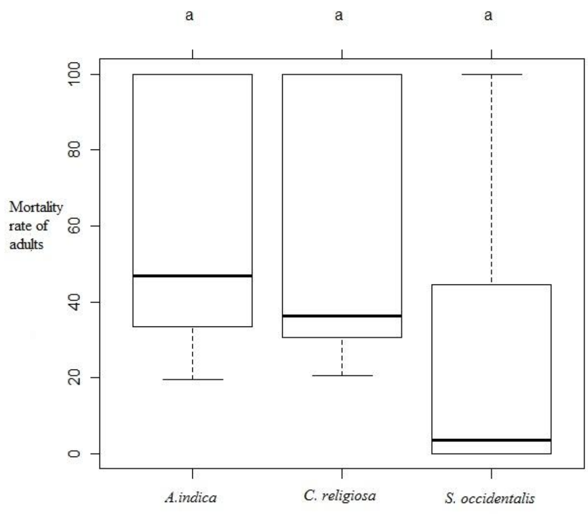
III.2. Adulticidal effect of different plants per dose.

The comparison of adult corrected mortality of *C. maculatus* subjected to the dose testD_1 (0.00273 g / cm^3) is presented in Figure 21. All plants gave mortalities from the first day of contact except S Westalis. *A. indica* and *C. religiosa* showed the same rate of mortality, with a greater efficiency of *A. indica*. This trend was reversed on the sixth day of contact and the last three days of testing. *S. occidentalis* gave mortalities only at ninth and tenth days of application. The FIG. 20 analysis shows a greater adulticidal efficacy of *A. indica* in the first five days of application with D_1, reversed tendency in favor of *C. religiosa* from the eighth day of contact. Throughout the experiment, *S. occidentalis* was found to be less effective than other plants. *S. occidentalis* produced adulticidal effects different from those produced by other plants, which gave similar effects to p <0.05 with the application of D_1.



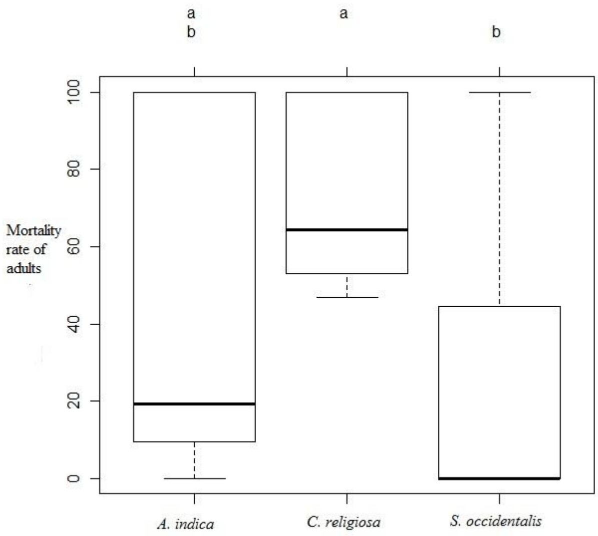
Adults mortality by the contact test D_1 (0.00273 g / cm^3)

D_2 (0.00546 g / cm^3) induced adult mortality with both plants (*A. indica* and *C. religiosa*) from the first day of contact with a higher potency of *C. religiosa*. Between the second and fifth day of contact, we observe more severe mortality with *A. indica*. *S. occidentalis* began to induce adult mortalities on the sixth day of application. These mortalities amount to 33.33% and are confused with those induced by *A. indica* on the same date; at the time when *C. religiosa* caused adult mortalities of 41.18%. A maximum efficacy of 100% is observed in both plants (*A. indica* and *C. religiosa*) from the eighth day of contact, whereas it can only be observed with *S. occidentalis* on the tenth day of application. Figure 21 summarizes a greater efficiency of *A. Indica* in the first five days of contact. This pace is reversed between the sixth and seventh days of the experiment with *C. religiosa*. As for D_1, *S. occidentalis* always exhibits a least adulticidal effect compared to other plants. The three plants showed the same impacts with the application of D_2 at p <0.05.

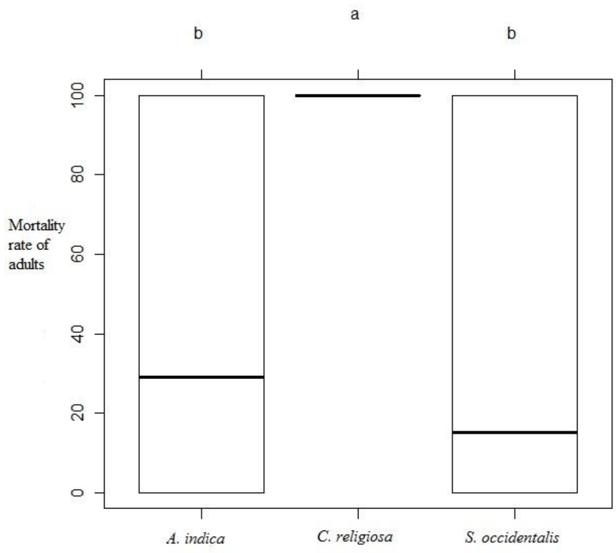


Adults mortality by the contact test D_2 (0.00546 g / cm^3)

The contact of crushed fresh leaves shows, with D_3 (0.0109 g / cm^3), a more noticeable adulticid efficacy with *C. religiosa* from the first day of application. This tendency persists until the seventh day of contact when this same plant induced 100% mortality. During which time the other plants, *A. indica* and *S. occidentalis* respectively gave 88.67% and 0% mortalities. *A. indica* shows maximum adulticidal efficacy on the eighth day of application, whereas it is observable only in the ninth with *S. occidentalis*. Figure 22 still shows a less important adulticidal efficacy of S. *occidentalis* and greater adulticide activity of *C. religiosa* throughout the duration of the tests. *A. indica* gave effects that are similar to those of other plants, which in turn induced different effects at p <0.05.



Adults mortality by the contact test D3 (0.0109 g / cm3)



Adults mortality by the contact test (D4 (0.0218g / cm3)

C. religiosa was very effective (100% mortality) from the first day of contact; at the time when *A. indica* and *S. occidentalis* gave respectively 2.25 and 5.18% mortalities. The effect did not begin to be felt until the sixth day of application with 55.33% adult mortality with *A. indica* and 44.33% adult mortality with *S. occidentalis*. All These plants showed increased adulticidal effects from the eighth day of contact. *C. religiosa* has distinguished itself from other plants with a maximum efficacy of 100% mortality from the first day of contact. While other plants (*A. indica* and *S. occidentalis*) showed this efficacy only on the eighth day of the experiment. These lasts have almost all given the same efficiency for each level of the application. With this dose, *C. religiosa* gave different effects from those induced by *A. indica* and *S. occidentalis*, which in turn produced the same adulticide impact with D_4 at $p<0.05$.

IV. Discussion

The contact of crushed fresh leaves of several plants was tested on eggs and adults of *C. maculatus* in the laboratory in ambient conditions. The plants showed differential efficiencies according to their nature and the applied dose. Thus, *A. indica* showed remarkable ovicidal efficacy (between 96.12 and 100%) with the application of all the doses. Statistically, we observed an equal efficiency of the effect of all doses at $p <0.05$. The ovicidal effect observed in C. religiosa reports deaths ranging from 73.33% to 90%. Thus the lowest dose (D1) and the highest dose have the same mortality rate of90% for*C. maculatus*eggs. *S. occidenalis* was found to be the less effective of all plants with a mortality exceeding not even 45% whatever the applied dose. The mortalities of the eggs induced by the contact of crushed fresh leaves of this plant are thus between 33.3% and 45%.
The lowest dose (D_1 (0.00273g / cm^3)) was found to be more effective on eggs than the two immediately higher doses (D_2 (0.00546g / cm^3) and D_3 (0.0109 / cm^3) whatever the applied plant. It also induced greater mortalities than those shown by the higher dose (D_4 (0.0218g / cm^3)) with the application of *S. occidentalis*. On the other hand, it gave the same effects as the latter with the application of each of the two other plants (*A. indica* and *C. religiosa*). The adulticidal activity of the contact of crushed fresh leaves showed slack mortalities of all doses with the application of all plants, for long. Thus, regardless of the used plant, we notice that the induced effects increase over time for each applied dose. We also noted that for all applied plants,

induced mortalities are dose dependent. With the lowest dose, *A. indica* was found to be more effective than theother plants at the first days of application, whereas the last few days were dominated by adult mortality of this insect induced by *C. religiosa*. The same trend is observed with dose D_1. On the other hand, for the other doses, we notice an inversion of situation thanks to *C. religiosa*.

Several authors using plants to control pests have shown the efficacy of several plants including those we used. Our results are in the same vein as those of Sarr (2010), who used one of the plants we tested, *C. religiosa* to show its effect on *Dermestes spp* with larger doses than which we used. It had recorded 100% mortalities only at the 34th day of application, whereas we recorded it at the 12th hour of application with the highest dose. . Furthermore, Mbaye *et al.* showed mortalities of adults of *Dermestes ssp* not exceeding 50% at the end of the day days 13 of application with the powder of leaves of C. religiosa (8g / 2kg)[12]. One might think that these differences are due to the difference in size between these two insects. We note that adults of *C. maculatus* are more sensitive to the application of *C. religiosa* than those of *Dermestes ssp*. The other plants we used also showed greater efficacy on the adults of *C. maculatus* than that highlighted in the work of this author. It thus appears that, in addition to that, the observed difference in efficacy would be linked to the difference in size of the insects, to the active molecules contained in these plants. Kellouche and Soltani have obtained significant mortality of adults of *C. maculatus* with the application of leaf powders from several plants such as *Ficus carica* (Moraceae) *Eucalyptus globulus* (Myrtaceae), *Olea europea* (Oleaceae), *Citrus limon*(Rutaceae) and *Syzygium aromaticum* (Myrtaceae)[2]. For this purpose, powders of *C. Limon*, *E. globulus* and *O. europaea* reduce the number of emerging adults by more than 50% at 4 and 5% doses. Their results also showed adults mortality of 100% in less than 24 hours with *S. aromaticum*. The biological activity of several other plants was tested, in powder, on adults of the main insect pest stored grain such as *C. maculatus* by many other authors. For example, the leaves and seeds of *Azadirachta indica* (Meliaceae) and the leaves and fruits of *Boscia senegalensis* (Capparidaceae) cause 80-100% Mortality of adults of *C. maculatus,* at doses between 2 and 4%[9]. Singh in 2011 showed the efficacy of *Ocimum sanctum* and Curcuma longa leaf powder on the oviposition of *C. maculatus*. Thus with the application of 0.5m G powder of these plants over 100 mg gave an oviposition of 45.64% *C. maculatus* with the application of *Ocimum sanctum* and of 40.92% *C. maculatus* with the application of *Curcuma longa*. This author has therefore shown that the effect of plants with biological activities is not only fatal but may affect the fecundity of insects such as *C. maculatus*. Application of *Securidaca longepedunculata* leaf powder on cowpea seeds at 5-10% (P / P) reduces or inhibits the emergence and damage of *C. maculatus* (F.)[8].

Comparing our findings with those of authors listed here, we can see the importance of native plants in the control of stored pest pests. To lighten the analysis of all these results, we note the development of certain plant species which seemed unnecessary but which in reality require a major importance in defending of the crops. In this context, the plants we have used are reinforced for their exploitation.

V. Conclusion:

Our study tries to set an effective formulation up against the pests of the stored products and applicable without any danger for the peasants. Thus the contact of the fresh ground leaves of C. religiosa, *A. indica* and *S. occidentalis*, indigenous plants of Senegal, was tested on the eggs and adults of cowpea beetle in the laboratory. On adults, our study states that *C. religiosa* is more effective than other plants on adult mortality of *C. maculatus* with the application of higher doses (D_2, D_3 and D_4).A. in another hand, for the lowest dose (D_1), *A. indica* was more aggressive on adults of this beetle, during the first seven days of the tests. It appears that, from all theseplants, *S. occidentalis* is the least effective plant in reducing the number of killed adults. As far as the eggs of this insect are concerned, they are more sensitive to the broth of leaves of *A. Indica* than those of the other plants. The crushed leaves of *S. occidentalis* are less effective on the eggs of *C. maculatus*. We also notice that the sensitivity of the external forms of this insect is different according to the applied doses. This formulation is very effective on the external forms of the greatest devastation of cowpea stocks. Thus, farmers will be able to protect their stock against this insect and possibly against other beetles that swarm in food stocks by the combining application of these three plants. To support the possibilities of storing foods such as cowpeas without exposing farmers to anydanger or worrying about damage caused by pest insects, we propose to test other formulations against these pests.

References

[1] A. Bell, O. Muck andH. Schneider.La protection intégrée des denrées stockées estune affaire rentable! GTZ, Eschborn, Germany, 1998. p 37.

[2] A. Faye, C. Thiaw, A. G. Ndiaye, M. Sembène. First investigation of different *Crateava religiosa* Forst formulations on the cowpea (*Vigna unguiculata* Walp.) seed-beetle, *Callosobruchus maculatus* Fabricius. *International Journal of Science and Advanced Technology*, *2 (8)*, 2012, 56-65

[3] A. Faye, M. Sarr, A. Samb, C. Thiaw, M. Sembène. Determination of impact of Azadirachta indica L. leaves on adults and eggs of Callosobruchus maculatus Fabricius, the largest predator of stored cowpea (Vigna unguiculata Walp) by an applicable method by farmers. *South Asian J Exp Biol*, *4 (5)*, 2014, 215-221.

[4] A.Faye, C. Thiaw, M. Sarr, M. Sembène.Effectiveness of different formulations leaves of *Senna occidentalis* on the external stages of *Callosobruchus maculatus* Fabricius main pest of cowpea (*Vigna unguiculata* Walp) stored. *International Journal of Biosciences 4(9)*,2014,246-253.

[5] A. Kellouche, N. Soltani. Activité biologique des poudres de cinq plantes et de l'huile essentielle d'une d'entre elles sur *Callosobruchus maculatus* (F.). *International Journal of Tropical Insect Science24(1)*, 2004, 184-191.

[6] A. P. Ouedraogo, S. Sou, A. Sanon, J. P. Monge, J. Huignard, M. D. Tran et P. F. Credland. Influence of temperature and humidity on populations of *Callosobruchus maculatus* (Coleoptera: Bruchidae) and its parasitoid *Dinarmus basalis* (Hymenoptera: Pteromalidae) in two zones of Burkina Faso. *Bull. Entomol. Res.86*, 1996, 695–702.

[7] C. Thiaw, A. G. Ndiaye, A. Samb, M. Sembène**.** Ovicid and adulticid effects of powders and extracts of *Calotropis procera* AIT. and of *Senna occidentalis* L. on *Caryedon serratus* (OL.) destroyer of groundnut stocks. *J. Sci. 7 (3)*, 2007.

[8] D. Seck.*Développement de méthodes alternatives de contrôle des insectes ravageurs au Sénégal par l'utilisation de plantes indigènes.* Thèse de Doctorat, Faculté des Sciences Agronomiques de Gembloux, 1994, 192

[9] D. Seck , G. Lognay, E. Hambrudge, M. Marlier, C. Gaspar et M. Severein. Biolocal activity of the shurb *Boscia senegalensis* Lam. (Capparidaceae) on stored grain insects. *J. Chem. Ecology, 29*, 1993, 377-389.

[10] J. A. Wahedi, L. D. David, A. Edward, B. P. Mshelmbula, & Z. Bullus. Efficacy of seed powder and extracts of *Azadirachta indica* L. (meliaceae) at graded levels on adult *Callosobruchus maculatus* (coleoptera: bruchidae) inMubi, north-eastern Nigeria. *International Journal Science and Nature.,4(1)*, 2013, 138-141.

[11] M. Kiendrebeogo, A. P. Ouedraogo, O. G. Nacoulma.Activités insecticides de *Strigahermonthica*(Del) Benth(*Scrophulariaceae*) sur *Callosobruchus maculatus* (Fab) (Coléoptera : Bruchideae). *Biotectnol. Agron. Soc.Environ. 10 (1)*, 2006, 17-23.

[12] N.N Mbaye, M. Sarr, A. G. Ndiaye, A. Samb, M. Sembène, Repulsive and biocide activities of leaves powder of Crataeva religi-osa (Forst) on Dermestes spp. associated with the salty smoked-dried fish. *International Journal of Biosciences4 (1)*, (2014), 306-312.